

**UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA
FACULTY OF MEDICINE**

**CHARACTERISTIC MODIFICATIONS OF THE
FETAL-MATERNAL INTERFACE IN
RECURRENT SPONTANEOUS ABORTION**

ABSTRACT OF DOCTOR'S DEGREE THESIS

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STATE OF KNOWLEDGE

Recurrent abortion (spontaneous recurrent pregnancy loss) is defined as the loss of three or more consecutive pregnancies. It affects almost 1% of all women, the risk of losing a pregnancy after three consecutive abortions being 55%. Many etiologies have been proposed for recurrent abortion but a vast number of authors consider 50% of them of unknown etiology.

For implantation to happen, the endometrium must be transformed into the decidua. This process consists in the modification of the endometrial stromal cells, the uterine glands, vessels, as well as the uterine immune system's cell population. In human beings, unlike other species, the process of decidualization is not dependent upon the presence of the blastocyst in the uterine cavity and starts in the late secretory phase of the menstrual cycle.

The preparation of the endometrium for the implantation is necessary and consists among other in establishing a presumptive window of implantation. In this period, the uterus is prepared for receiving a blastocyst and supporting a future implantation by mediating the immune cells, the cytokines, the growth factors, the chemokines and the adhesion molecules.

A critical step in the consolidation of pregnancy is the decidualization process when the endometrium suffers extensive modifications regarding the morphology as well as the expression and secretion patterns for supporting the blastocyst implantation.

The normal pregnancy has been considered as a biological example of semi-allogenic grephon acceptance, the semi-allogenic fetus being protected by the mothers' immune attack. It's interesting to notice that the so-called semi-allogenic product is, in fact, made of trophoblastic cells coming from the fetal-maternal interface.

Evaluating the first trimester spontaneous abortion includes following the morphological characteristics of the gestational sac, the embryo or the fetus and the placenta. An empty gestational sac does not allow for an evaluation of the embryo or the fetus. When embryo/fetus examination is possible development anomalies can be detected as well as growth restrictions or the presence of isolated morphological defects. Imagistic preejection techniques such as embryoscopy and high resolution sonography can aid in this evaluation.

PERSONAL CONTRIBUTIONS

Aims and objectives of the study

The present study is structured around the following major objectives:

1. Identifying cellular and molecular processes that can lead to a recurrent abortion
2. The correlation of these processes with pregnancy degradation echographic markers
3. The characterization of the implantation site by means of histological and immunohistochemical analysis.
4. The evaluation of angiogenesis and vasculogenesis around the implantation site in the normal pregnancy and in the recurrent abortion
5. Paraclinical evaluation by imagistic means of the evolution of the normal pregnancy and the recurrent abortion pregnancy.

Therefore I have conducted three ample researches upon the studied cases – normal pregnancies with induced abortion by request in the first trimester and the recurrent abortion cases studied.

1. Statistic analysis of the studied groups. The statistic analysis consisted in processing the experimental data by means of descriptive statistics, graphical representations, statistic inference tests and correlation studies of the parameters in the spontaneous abortion and the induced abortion upon request (representing a normal pregnancy).

2. Paraclinical evaluation of the normal pregnancy and the recurrent abortion pregnancy using imagistic methods. By corroborating the expression of the morphological modifications of the echographic aspects obtained with 2D and 3D transvaginal sonography with the maternal hormonal status and the risk factors we can establish an investigation algorithm for the cases in which spontaneous abortions occur, for limiting the risk of repeating the abortive event in future pregnancies.

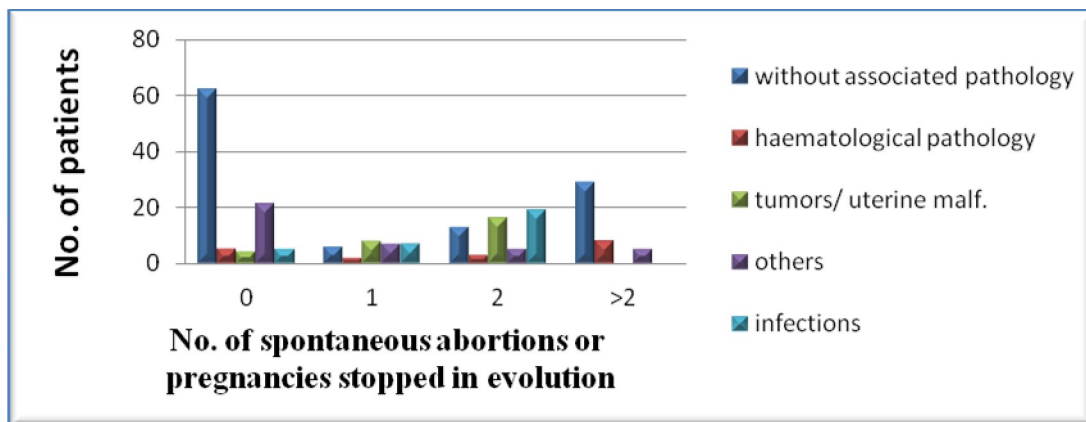
3. The characterization of the implantation site through histological and immunohistochemical exams. We wanted to evaluate the angiogenesis and the vasculogenesis using the immunohistochemical expression of CD31 and CD34, to evaluate the immune response looking for the presence of T lymphocytes – Th (the expression of CD8 marker) and Tm (the expression of CD3 marker), to evaluate the uNK cells, all this markers being correlated with the evolution of pregnancy.

Statistical analysis of the studied groups

Out of 226 cases with spontaneous abortion, we have identified those with recurrent spontaneous abortion (RSA). In this category we have included the patients with at least three spontaneous abortions in the past, thus splitting the group study into two smaller groups: one that included the 43 patients with RSA and the second one that included the 183 patients with a maximum of two spontaneous abortions in the past (non-RSA).

We have individualized a number of 29 cases of idiopathic recurrent spontaneous abortion leaving us with 14 cases of non idiopathic RSA. Correlating these 14 cases with the obstetrical antecedents of the patients we divided them into two more groups: primary RSA group (4 cases) – the patient never had any births in antecedents and secondary RSA group (10 cases) – the patient had one or more births in the past.

In the studied group with spontaneous abortions in the past, we have found a series of associated pathology, including non recurrent spontaneous abortion (less than three spontaneous abortions in the past). Out of a total of 226 patients with spontaneous abortion included in the group study, 110 didn't have any associated pathology. In this category we have included the subgroup of patients with idiopathic RSA (29 cases), the rest of the cases having a confirmed diagnostic.

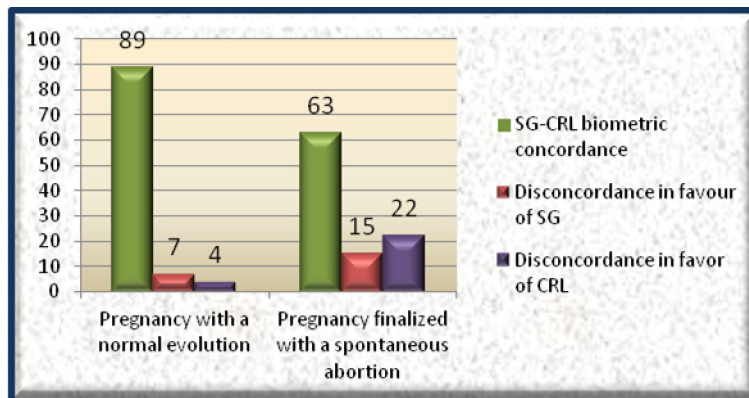


By comparing the average age of the two studied groups we have found a significant statistic difference between the average age of the patients in the study group compared to the standard group, with $p < 0.05$. This shows us that age is a marker with a potential influence in the recurrent abortion initiation.

Ecographic analysis of the studied cases

Regarding the growth rate of the gestational sac, in the standard group pregnancies we have found an average growth rate of 1.32mm per day (this value is considered normal), while in the study group with pregnancies finalized with a spontaneous abortion, the growth rate of the gestational sac was only about 0.63mm per day.

The values we have found in our study suggest that the difference between the sizes of the gestational sac and the cranial-caudal length of the embryo (5.5-7.5mm) can be considered an indication of a good prognostic for the further evolution of the pregnancy. A value situated outside the mentioned interval can represent an alarm signal, suggesting a less favourable prognostic.



In risk pregnancies the embryo's heart rate was between 72 and 106 beats per minute, bradycardia being a negative prognostic factor in the evolution of pregnancy.

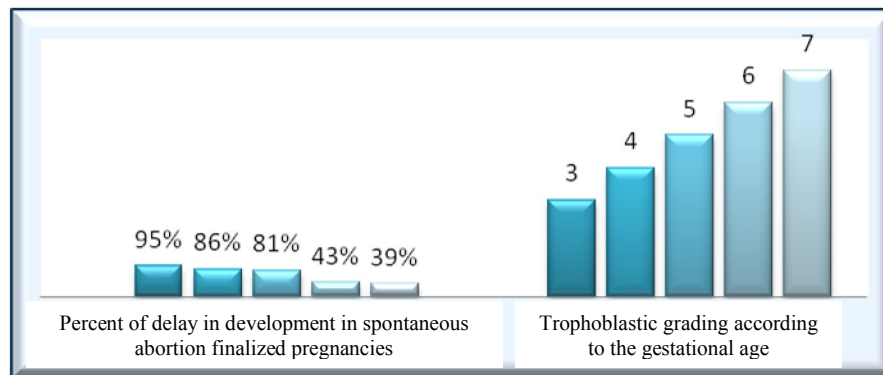
Elevated values of the trophoblastic thickness correlation parameter above 3 have been observed in a significant percentage of cases with spontaneous abortion – 68%, suggesting that the placentation defect, translated sonographically into a thin trophoblast is one of the most important causes of pregnancy loss. In the standard group, values above 3 of the trophoblastic thickness correlation parameter have been met only in 5% of the cases.

By analysing the data, we have found that there is a strong statistic correlation between the trophoblastic thickness correlation parameter and the spontaneous abortion in the first trimester.

Given the results, a value greater than 3 of the trophoblastic thickness correlation parameter can be seen as an indication for a closer monitoring of a pregnancy with a potentially unfavourable evolution.

Characterization of the implantation situs through histological and immunohistochemical exams

Following histological studies on biological trophoblastic material from normal pregnancies, we have established a histological grading of the trophoblast in accordance to the gestational age. In accordance to this histological gradient of a normal pregnancy, we have followed histological modifications that occurred at the implantation site in pathologic pregnancies with recurrent abortion. Delays of the histological development stadium have been observed in the histological evaluation of the sampled trophoblast from spontaneous abortion finalized pregnancies.



Following the CD8 expression in cases with spontaneous abortion, we have seen a weak expression of this immunohistochemical marker, these findings being in accordance to the data exposed in the literature.

The evaluation of angiogenesis and vasculogenesis has been done by means of immunohistochemical expression (CD31, CD34) and by assessing the expression of some tissue factors implicated in the reshaping of the implantation and endometrium angioarchitecture. In the RSA study group we have found 18 negative answers and 11 weak positive expressions in the spongy decidua and few CD31 positive vessels in the endothelial cells and inhomogeneous positive answers in the dilated capillaries.

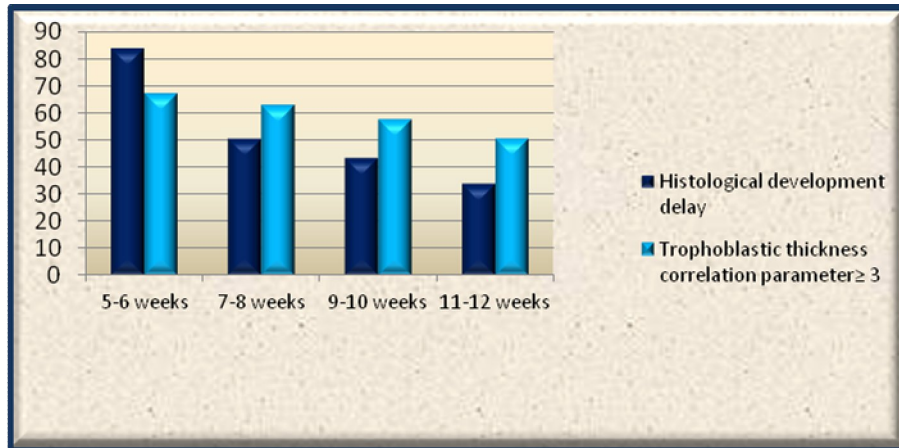
In the study group represented by the RSA, we have found a weak positive expression and even a positive expression of the CD34 in the vessels, in the great vessels as well as in the small colabated vessels, in the decidua's vessels near the necrosis areas and in the vessels of the spongy deciduas.

A characteristic of the VEGF expression in the RSA study group was the variable expression of the VEGF-C. This variability of the VEGF expression might lead to an imperfect vasculogenesis that can threaten the pregnancy's evolution.

In the RSA study group, the expression of CD56 was low in 71.24%. This can suggest that following the presence of this type of cells can be beneficial after a spontaneous abortion event, the issue being an altered endometrial immunity in patients with RSA.

Complex evaluation through clinical, echographic, histological and imunohistochemical methods of the idiopathic recurrent spontaneous abortion

We have noticed a good concordance of the data in the histological study with the trophoblastic thickness correlation parameter. This observation is valid for the pregnancies ending up with a spontaneous abortion after the 6th week of gestation. The smaller pregnancies have a lesser degree of concordance of the two parameters.



Concluding this study we have been able to establish a correlation between RSA and defective placental bed development because of inadequate trophoblastic invasion and limited maternal spiral arteries transformation. This data is in accordance with the studies in the medical literature. From a histological point of view, the trophoblastic layer is thin and discontinuous and the trophoblastic infiltration of the endometrial vessel lumen and the decidua is reduced or absent in the vast majority of RSA cases.

FINAL CONCLUSIONS

- In our study the incidence of the RSA was 19.02% out of the total of spontaneous abortion patients. The incidence of idiopathic RSA was 67.44% out of the total number of RSA patients and 12.83% out of the total number of patients with spontaneous abortion.

- The obtained values suggest that the difference between the size of the gestational sac and the cranial-caudal length of the embryo is between 5.5-7.5mm and can be considered a good prognostic for the evolution of pregnancy.

- The pregnancies that presented a correlation parameter of the thickness of the trophoblast greater than 3 require a close ultrasonic monitoring because of the increased risk of a spontaneous abortion. Higher values of the parameter of correlation of trophoblast thickness above 3 have been observed in a significant percentage of cases with spontaneous abortion – 68%.

- We have completed a grading of the trophoblast according to the gestational age with the help of the collected materials from the pregnancies with a normal evolution.

- By using the CD56 immunomarker in the two study groups we have managed to show a reduced expression of 71.24% of the biological samples collected from the pregnant women with spontaneous abortion opposed to 84.53% in the control group. Following the expression of CD8 in cases with spontaneous abortion we have observed a decreased expression of this immunohistochemical marker in 69.27% of the cases.

- Using CD31 and CD34 immunomarkers we have observed the prevalence of a reduced expression in the biological data collected from the pregnant patients with spontaneous abortion, in a predominant proportion: CD31 immunomarking – reduced expression in 72.34% of cases; CD34 immunomarking – reduced expression in 69.32% of cases.

- When using VEGF antibody immunomarker we have observed a variable positivity in distribution as well as intensity. This variability of the VEGF expression can lead to the imperfect vasculogenesis that can determine an interruption in the course of pregnancy.

- By correlating all this elements present in a compromised pregnancy, the complex studies we have conducted entitle us to establish a histological grading of an abortion compromised pregnancy. This grading can be used to establish a presumptive cause of pregnancy loss and to initiate a new classification of the elements involved in the placental vasculogenesis in the first trimester of pregnancy.